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IMPACT OF SELENIUM ON GUT INFLAMMATION


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# ABSTRACT

Ulcerative colitis (UC) is a chronic condition that causes inflammation in the colon. Because there are only temporary solutions to UC, there is a significant interest within the biomedical community to discover preventive as well as permanent treatment measures for it. During an inflammatory response in the gastrointestinal tract, the composition of the microbiota has been shown to change, subsequently creating an environment where pathogenic microbes can colonize and increase the probability of a relapse. Due to its role in mitigating inflammation and affecting immune cell function, the micronutrient selenium offers a potential solution for managing the dysregulated immune response seen in inflammatory bowel disease (IBD). This dissertation examines several experiments involving gut inflammation and the ability of selenium in modulating inflammation at several levels in mice on respective selenium diets that are deficient (<0.01 ppm selenium), adequate (0.08 ppm selenium), or supplemented (0.4 ppm selenium). The effects of selenium on bacterial growth *in vitro*, bacterial virulence *in vivo*, and microbiota composition in a chemical (dextran sodium sulfate, DSS)-induced colitis murine model will be investigated. These studies are based on the hypothesis that mice on selenium supplemented diets will have minimal changes in their microbiota composition and will resolve the inflammatory condition faster than mice placed on selenium deficient or selenium adequate diets. If successful, these experiments could provide further insight into preventive and maintenance treatments in patients affected by IBD.

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## Chapter 1

## Introduction

**Selenium**

Selenium is an essential micronutrient that has important antioxidant, anti-inflammatory, and chemopreventive roles in the body (Papp, L.V. et al., 2007; Kaushal, N., 2012). These physiological functions of selenium are attributed to its incorporation into selenoproteins (Hoffmann, P.R., 2007). The two main groups of selenoproteins include glutathione peroxidases (GPx1, GPx2, GPx3, GPx4, and GPx6), which mediate oxidative stress in cells by decreasing levels of reactive oxygen species (ROS) using glutathione, and thioredoxin reductases (TR-1, TR-2, and TR-3), which reduce thioredoxin (Papp, L.V. et al., 2007; Hoffmann, P.R., 2007). A third group, Rdx, has recently been discovered, and includes SelH, SelT, SelV, and SelW (Dikiy, A., 2007). Despite these discoveries, more research is needed to elucidate the functions of selenoproteins (Hoffmann, P.R., 2007; Kaushal, N., 2012).

Selenoproteins incorporate selenium in the form of selenocysteine through a biologically conserved process (Papp, L.V. et al., 2007). Selenocysteine (Sec) is considered to be the $21^{st}$ amino acid and is coded for by the UGA codon (Papp, L.V. et al., 2007). Briefly, in Sec biosynthesis seryl-tRNA$^{[Ser]Sec}$ is phosphorylated and the serine is displaced by Sec, yielding Sec tRNA$^{[Ser]Sec}$; the selenocysteine insertion sequence (SECIS) element, an RNA hairpin structure in the 3'-untranslated region (UTR), is needed to initiate the decoding of the penultimate UGA codon for Sec (Papp, L.V. et al., 2007). The selenocysteine redefinition element (SRE), SECIS-

binding protein 2 (SBP2), and Sec-specific elongation factor (EFSec) are other components that

help facilitate Sec incorporation (Figure 1) into selenoproteins (Papp, L.V. et al., 2007).
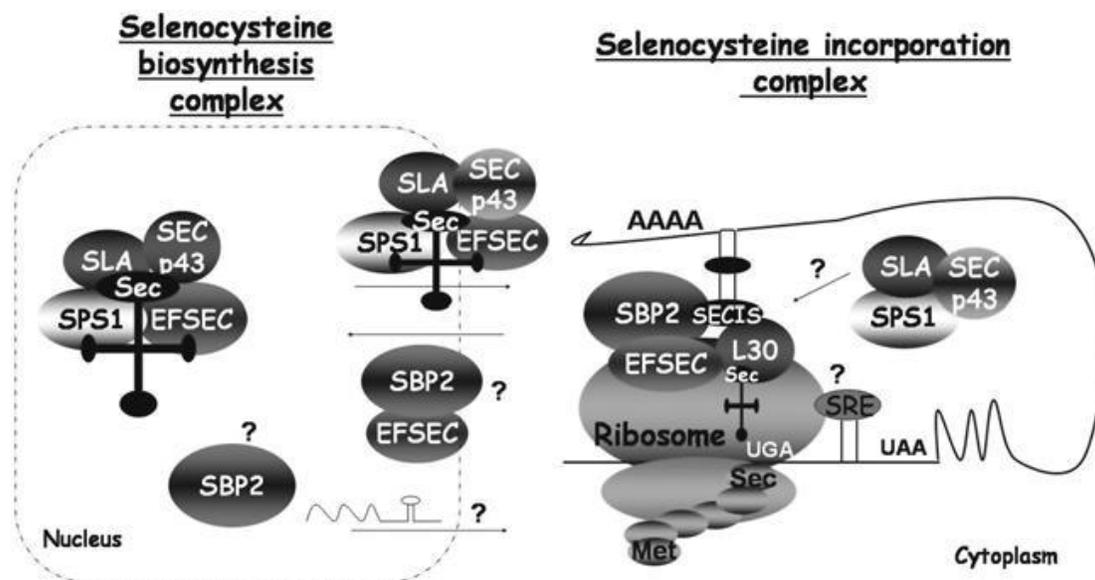


**Figure 1. Mechanism for Sec biosynthesis and incorporation into selenoproteins** (Image from L.V. Papp et al., 2007).

**Antioxidant properties of selenium**

The antioxidant properties of selenium can be attributed to its ability to mediate oxidative

stress by reducing reactive oxygen and nitrogen species (RONS) in cells (Kaushal, N., 2012).

Research has also shown that selenium plays an important role in mitochondria by upregulating

the key transcription factors nuclear respiratory factor-1 (Nrf-1) and mitochondrial transcription

factor-A (mtTFA) (Tirosh, O., 2007). Upregulation of these mitochondrial transcription factors

may contribute to mitochondrial redox regulation, effectively decreasing the amount of ROS

produced (Kaushal, N., 2012). Thus, selenoproteins can have effects through redox-dependent

pathways that could potentially impact immune responses and other physiological processes.

**Selenium and the immune response**

Recent studies have shown that selenoproteins play an integral role in the immune response, especially in macrophages and T cells (Kaushal, N., 2014; Carlson, B.A. et al., 2010). Genetic knock out of Sec tRNA[Ser]Sec (*Trsp*) showed that the absence of selenoproteins in T cells effectively inhibited T cell maturation and effector functions as well as their capacity to mediate their production of ROS (Carlson, B.A. et al., 2010). The absence of selenoproteins in macrophages mainly affected macrophage migration (Carlson, B.A. et al., 2010). Interestingly, dietary selenium supplementation has been shown to have physiological effects on macrophages, resulting in a class-switch from the classically-activated and pro-inflammatory (M1) macrophage phenotype to the alternatively-activated and anti-inflammatory (M2) macrophage phenotype (Nelson, S.M., 2011). The M2 macrophages dampen inflammatory signals and, thus, facilitate pathways of resolution of inflammation (Nelson, S.M., 2011).

**Selenium and Ulcerative Colitis**

Ulcerative colitis (UC) is one of the most prevalent types of inflammatory bowel disease (IBD), affecting 7.6 to 246.0 people/100,000 per year, and is characterized by chronic inflammation of the colon and rectum (Gentschew, L. and L. Ferguson, 2012; Danese, S. and C. Fiocchi, 2011). Unlike UC, which is mostly restricted to the colon, Crohn's disease (CD) can affect any area of the gastrointestinal tract and often has dispersed inflammation (Gentschew, L. and L. Ferguson, 2012).

The exact causal mechanism for UC remains unclear; however, diet, genetics, environmental factors, dysregulated immune responses, and the host's gut microbiota are all

thought to play a role in its development (Noor, S. et al., 2010; Lepage, P. et al., 2011; Gentschew, L. and L. Ferguson, 2012). Current treatments with aminosalicylates, steroids, and immunosuppressive drugs for UC are focused on remission, but they are not conducive for the long-term given the potential side effects associated with these drugs (Danese, S. and C. Fiocchi, 2011). Surgery is the closest definitive option for patients with UC, although the colectomy could induce a secondary infection leading to further complications (Danese, S. and C. Fiocchi, 2011).

**Experimental Colitis Models**

In this work, two models of gut inflammation were utilized to mimic chemical injury or bacterial injury (via changes in the gut microbial community structure) of the gut that are one of the several likely etiological factors known to cause colitis.

*Citrobacter rodentium model*

*Citrobacter rodentium* is a natural enteric pathogen in mice that is related to *Escherichia coli*, yet it is not pathogenic in humans (Mundy, R., 2005). Its attaching and effacing (A/E) lesion pathology and induction of a Th1 response in the gastrointestinal tract make *C. rodentium* a good model for IBD (Mundy, R., 2005).

**Dextran sodium sulfate (DSS)-induced colitis**

The polysaccharide DSS provides another model for IBD, specifically UC due to its distal colon pathology, disruption of the intestinal mucosal barrier, and induced inflammation (Laroui, H. et al., 2012). Previous studies done in our laboratory have shown that selenium supplementation in mice can help M2 macrophages protect against DSS-induced colitis (Narayan, V. et al., 2015)

**Gut Microbiota**

The microbiota in the gastrointestinal tract (GI) provide many essential and beneficial roles for their hosts, including facilitating digestion and absorption, priming and maintaining successful immune functions, aiding in the turnover of intestinal epithelium, and preventing pathogen colonization of the gut (Neuman, M.G. and R.M. Nanau, 2012). There are four main bacterial phyla that colonize the human gut: *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* (Frank, D.N. et al., 2007). *Firmicutes* constitutes bacteria from orders such as *Clostridiales,* while bacteria of the order *Bacteroidales* are from the phylum *Bacteroidetes* (Frank, D.N. et al., 2007). The suborder *Actinomycinaeae* and the family *Enterobacteriaceae* belong to the phyla *Actinobacteria* and *Proteobacteria*, respectively.

*Inflammatory bowel disease and the gut microbiome*

One of the causative factors associated with IBD is marked by dysbiosis, or a change in the natural gut microbiome that increases the proportion of pathogenic bacteria in the GI tract

(Neuman, M.G. and R.M. Nanau, 2012). In healthy individuals, the most prevalent bacteria belong to the phyla of *Firmicutes* and *Bacteroidetes*, and dysbiosis shifts this ratio, increasing the *Actinobacteria* and *Proteobacteria* in CD or UC (Frank, D.N. et al., 2007). Interestingly, pathogenic bacteria are thought to invade and colonize the gut by initiating the host's inflammatory response, which helps to facilitate dysbiosis (Stecher, B. and W. Hardt, 2008).

*Selenium and the gut microbiome*

Studies comparing selenium levels between germ-free and conventional mice have shown that bacteria in the gut use some of the host's selenium (Hrdina, J. et al., 2009; Kasaikina, M. et al., 2011). Additionally, dietary Se supplementation has been found to increase bacterial diversity in the GI tract (Kasaikina, M. et al., 2011).

**Statement of the Problem**

Selenium is known to be an important modulator in inflammation due to its antioxidant and anti-inflammatory properties. IBD is an example of a chronic disease with persistent inflammation, yet its exact cause and pathophysiology remain unknown. Because selenium has been shown to have beneficial effects on downregulating inflammation, facilitating immunocompetence, and colonization of gut microbiota, its collective effects were investigated on IBD. The specific hypothesis of this research was that *dietary selenium supplementation would help maintain normal function of the gut due to decreasing the duration of the infection, decreasing the enteropathogenic virulence, and maintaining the natural gut flora during induced inflammation*. Understanding how dietary selenium supplementation can be used to mediate

inflammation in chronic diseases like ulcerative colitis and Crohn's disease will provide insight into more cost-effective and population-based preventive treatments compared to the expensive individualized treatments that are currently available.

**Specific Aims**

This study was performed with the following research objectives:

1. To investigate the effect of selenium on *C. rodentium* growth *in vitro*. This will be further studied in mice looking at whether the absence of selenium in the growth media prior to inoculation will decrease bacterial virulence *in vivo*.

2. To investigate if selenium supplementation can decrease or prevent dysbiosis of the gut microbiota during inflammation.

# Chapter 2

# Materials and Methods

## Mice and Diet

Twenty-eight C57BL/6 mice were used in these experiments. Sixteen mice were used for the *Citrobacter rodentium* infection and placed on selenium deficient diets for at least 8 weeks before infection. The mice were inoculated with either *C. rodentium* cultured in selenium deficient media or those cultured in 250 nM sodium selenite (n=8 per group). All 16 mice were housed in separate cages to prevent them from transmitting the infection to each other.

For the dextran sodium sulfate (DSS) model, twelve mice were used. The mice were placed on either selenium deficient (<0.01 ppm Se; n=3), selenium adequate (0.08 ppm Se; n=3), or selenium supplemented (0.4 ppm Se; n=3) diets for at least 8 weeks. This is a standard method that has been optimized in our laboratory to deplete or replete selenoproteins *in-vivo*.

## Culturing *C. rodentium*

*Citrobacter rodentium* was a kind gift from Dr. Margherita Cantorna's lab (Strain ICC 169, nalidixic acid resistant to 50µg/mL). *C. rodentium* was cultured in M9 minimal salts media (Difco M9 Minimal Salts, 5×) before all experiments to ensure that no extraneous selenium or other micronutrients were present (Table 1). Selenium (as sodium selenite) was added to the M9 minimal salts media accordingly in each experiment.

| Nutrient | Approx. contents per 300mL |
|---|---|
| Disodium Phosphate (anhydrous) | 10.17g |
| Monopotassium Phosphate | 4.5g |
| Sodium Chloride | 0.75g |
| Ammonium Chloride | 1.5g |
| Glucose (20% solution) | 6 mL |
| 1M $MgSO_4$ | 0.6 mL |

**Table 1. Components of M9 minimal salts media**

### *C. rodentium* **growth** *in vitro*

Six test tubes were prepared with 15mL M9 minimal salts media, 1mL *C. rodentium* (cultured overnight in M9 minimal salts media), and 4mL Milli-Q water. Sodium selenite (Sigma Chemicals) was added to all but one test tube to make concentrations of 0, 50, 100, 250, 500, or 1000 nM selenium. The test tubes were incubated for 21 hours at 37°C at a low rpm (New Brunswick Scientific C24 Incubator Shaker). Optical density (OD) of each test tube was taken with a UV-Vis spectrophotometer at 600 nm in half-hour intervals for the first 15 hours and in hour intervals after that.

### *C. rodentium* **infection**

Mice were inoculated with *C. rodentium* via oral gavage according to the *C. rodentium* inoculation protocol from Dr. Margherita Cantorna's lab (Varshney, J. et al., 2013), except that M9 minimal salts media was used instead of LB broth in order to avoid exogenous selenium contamination. Before the inoculation, *C. rodentium* was cultured overnight in two different flasks, one in the absence of selenium (0 nM Se) and the other in the presence of selenium (250

nM Se). Each mouse was then inoculated with 200 µL of the inoculum ($5\times10^9$ CFU). Fecal

samples were collected every 2-3 days to observe the status of the infection.

**Plating *C. rodentium***

Fecal pellets that were collected from the infected mice were plated as per the protocol

from Dr. Margherita Cantorna's lab to observe the status of the bacterial infection. Plates were

prepared using LB agar (Novagen) and adding 50µg/mL nalidixic acid, an antibiotic, to inhibit

the growth of other bacteria on the plate and stored upside down at 4°C to reduce condensation

forming on the agar. After collection, the fecal pellets were weighed (at least 0.025g) and then

immediately diluted with sterile phosphate buffered saline (PBS) in a ratio of 0.1g murine

feces/mL PBS to prevent the bacteria from dying. The samples were homogenized and 1:10

serial dilutions in PBS were performed as needed. The diluted homogenate was vortexed briefly

and plated in triplicate (25µL/plate), and at least three dilutions were plated. A Bunsen burner

was utilized throughout the homogenization and plating to minimize any contamination. The

agar plates were then incubated overnight at 37°C and the *C. rodentium* colonies were counted

the next day. Plate dilutions aimed to get between 10 and 100 colonies per plate. CFU/g feces for

*C. rodentium* fecal shedding was calculated according to the following equation:

$$\left(\frac{\text{Average number of colonies}}{0.025\text{mL}}\right) \times \text{Dilution Factor} \times \left(\frac{0.1\text{mL PBS}}{0.1\text{g feces}}\right) = \textbf{CFU/g feces}$$

**DSS-induced Colitis**

Selenium deficient mice were given 4.0% DSS in their drinking water to chemically induce a colitis model. The drinking water was changed on days 2 and 4 and removed permanently on day 5. Fecal samples were kindly provided by Dr. Naveen Kaushal and stored at -80ºC. The mice were sacrificed on day 10.

**DNA Isolation**

Several different genomic DNA isolation techniques were employed to obtain suitable results due to downstream inhibition of PCR: Mo Bio PowerLyzer PowerSoil DNA Isolation Kit, phenol:chloroform:isoamyl alcohol (25:24:1) extraction, and QIAamp DNA Stool Mini Kit. DNA isolation using Mo Bio PowerLyzer PowerSoil DNA Isolation Kit and QIAamp DNA Stool Mini Kit were performed followed as per their protocols. An additional DNA isolation protocol was adapted from Zhang et al. (2006). Fecal samples were weighed, vortexed, washed with ethanol, and centrifuged. The supernatant was discarded and the remaining part washed with TE buffer, then TNE (10mM Tris-Cl, 0.5% SDS, and 1mM $CaCl_2$) and Proteinase K was added and the tube incubated for approximately 2 hours. The supernatant was transferred to a new centrifuge tube with potato starch, vortexed, incubated, and supernatant again removed. A high concentration salt solution (3.5M NaCl) was added, followed by incubation and subsequent extraction with phenol:chloroform:isoamyl alcohol (25:24:1). The supernatant was taken and 4M guanidine hydrochloride and 1M potassium acetate were added. A spin column was used to wash and then elute the DNA in TE buffer.

**DNA Purification**

DNA was purified using several methods: Mo Bio PowerClean Pro DNA Clean-Up Kit, purification with isopropanol (Sigma Chemicals), and purification with lithium chloride (Viennois, E. et al., 2013).

Standard isopropanol protocol was used to remove any potential PCR inhibitors. Briefly, an equal volume of isopropanol was added to the DNA, incubated for 60 minutes at -20°C, centrifuged, and the pellet was washed with 70% cold ethanol and allowed to dry before re-suspending in TE buffer.

The protocol for lithium chloride purification to remove DSS from nucleic acid samples was adapted from Viennois et al. (2013). The protocol was slightly modified since DNA had already been isolated. Briefly, 0.1 volume LiCl was added to isolated DNA sample, incubated on ice for 2 hours, centrifuged, and the pellet was re-suspended in TE buffer. The previous steps were repeated, and the subsequent pellet was washed with ethanol and re-suspended in TE buffer.

**Agarose Gel Electrophoresis**

DNA samples were run on a 1% agarose gel for 35 minutes at 100V to observe any DNA degradation due to the isolation techniques. Briefly, agarose (Denville Scientific) was mixed with Milli-Q water, and a 5μL of ethidium bromide was added before gel was poured into tray. After electrophoresis, the gel was visualized with a UV light on a transilluminator to observe DNA.

**Quantitative real-time PCR**

DNA from fecal pellets was amplified using quantitative real-time polymerase chain reaction (RT-PCR). Primer pairs and their specific 16S rRNA target sequences (Invitrogen) were designed for *Actinobacteria*, two classes of *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* (Table 2) based on primer pairs reported by Bacchetti De Gregoris et al. (2011). A control primer was designed as reported by Bacchetti De Gregoris et al. as well (2011).

**Table 2. Primer pairs used to identify bacterial phyla in the gut.**

| | | |
|---|---|---|
| **Actinobacteria** | **F** | TACGGCCGCAAGGCTA |
| | **R** | TCRTCCCCACCTTCCTCCG |
| | | |
| **Proteobacteria** | | |
| α-Proteobacteria | **F** | CIAGTGTAGAGGTGAAATT |
| | **R** | CCCCGTCAATTCCTTTGAGTT |
| | | |
| γ-Proteobacteria | **F** | CGTAAGGGCCATGATG |
| | **R** | TCGTCAGCTCGTGTYGTGA |
| | | |
| **Bacteroidetes** | **F** | CRAACAGGATTAGATACCCT |
| | **R** | GGTAAGGTTCCTCGCGTAT |
| | | |
| **Firmicutes** | **F** | TGAAACTYAAAGGAATTGACG |
| | **R** | ACCATGCACCACCTGTC |
| | | |
| **Universal** | **F** | AAACTCAAAKGAATTGACGG |
| (Control) | **R** | CTCACRRCACGAGCTGAC |

A master mix was made for each set of bacterial DNA primers comprised of 0.545µL of each forward and reverse primer (20µM stock) and 11.25µL PerfeCTa® SYBR® Green SuperMix, ROX (Quanta Biosciences). The optimal concentration of DNA used in PCR assay was experimentally determined to be 25ng/µL, although 100ng/µL DNA was initially used.

The reaction conditions for the PCR assay were adopted from the protocol developed by Bacchetti De Gregoris et al. for these bacterial phyla primers (2011). Briefly, the initial denaturing step was 95ºC for 5 minutes and 40 cycles consisting of 3 different steps: 95ºC for 15 seconds, 61.5ºC for 15 seconds, and 72ºC for 20 seconds.

# Chapter 3

# Results

## *C. rodentium* growth *in vitro*

Since bacteria contain selenoproteins, it was investigated whether culturing *C. rodentium* in fixed selenium environments would affect its growth. The particular bacterial species chosen was *C. rodentium* since it would be used in the subsequent *in vivo* experiments due to its natural pathogenicity in mice and potential as an IBD model.

*C. rodentium* displayed relatively similar growth through the third time point (1.5 hours), and leveled off by time point 18 (9 hours), regardless of the selenium concentration (0, 50, 100, 250, 500, and 1000 nM) of the media (Figure 1). The *C. rodentium* cultured in the two intermediate selenium media concentrations, 100 nM and 250 nM, had similar OD readings to each other except during the exponential growth stage, and they showed a higher OD than the others by the fourth time point, even after leveling off. Se deficient (0 nM) and highest selenium supplemented (1000 nM) cultures had the lowest OD readings. Lastly, the 50 nM and 500 nM selenium supplemented media had OD readings similar to each other and values were in between the other four cultures. The bacterial growth curve displayed in Figure 2 is from one of three trials (other data not shown), and it follows exponential bacterial growth, as expected.  In summary, at the mid log phase of growth, there appeared to be a dose-dependent effect of selenium on growth, with an increase up to 250 nM followed by a decrease at higher

concentrations. Such a Gaussian (normal) distribution suggests the ability of high levels of

selenium to potentially cause toxic effects in *C. rodentium* leading to decreased growth.
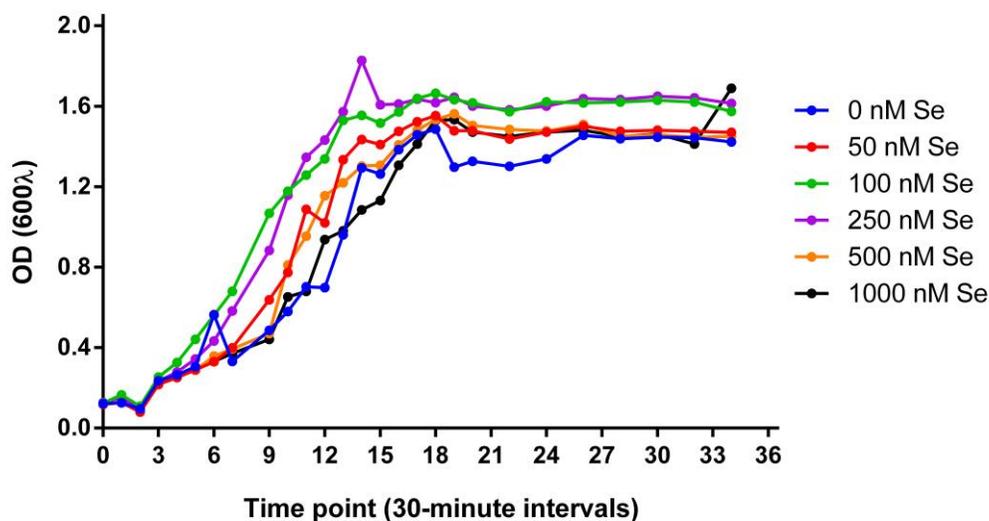


**Figure 2. Selenium levels affect *C. rodentium* growth *in vitro*.** Growth curve for *C. rodentium* cultured in various selenium supplemented (0, 50, 100, 250, 500, and 1000 nM Se) M9 minimal salts media (data shown from first trial).

### *C. rodentium* virulence *in vivo*

Given that *C. rodentium* growth was affected by selenium supplementation *in vitro*, it

was investigated whether the virulence of *C. rodentium* would be decreased *in vivo* in mice after

being cultured in selenium deficient media.

Two of the eight mice given the selenium deficient *C. rodentium* innoculum died within

the first 48 hours. To determine if this was caused by the innoculation process or sepsis, the

airway was examined for possible punctures and samples of the spleen and peritoneal fluid were

plated. For one mouse, the plates grew too many colonies to count (TNTC) from both the spleen

and peritoneal fluid, and for the second mouse, the plates had $1.1\times10^{10}$ CFU/mL from the spleen

and $4.2\times10^3$ CFU/mL from the peritoneal fluid.

Based on the averaged data of the two groups of mice (Figure 3), the infection lasted 9

days longer than the expected 21 days for both groups, with a slight increase in bacterial

shedding on day 26. Mice inoculated with the selenium deficient *C. rodentium* exhibited slightly

lower bacterial fecal shedding until day 21 with a peak in bacterial shedding on day 10. This

differed from the observed bacterial shedding in mice innoculated with the selenium

supplemented *C. rodentium* that had an average peak on day 5 and a decrease in relative bacterial

shedding after day 21. These studies will need to be repeated with *C. rodentium* cultured at

higher concentrations (500 nM) of selenium to examine if such a "bacteriocidal" effect has any

impact on the bacterial colonization and possible differences in the pathogenesis of mice.
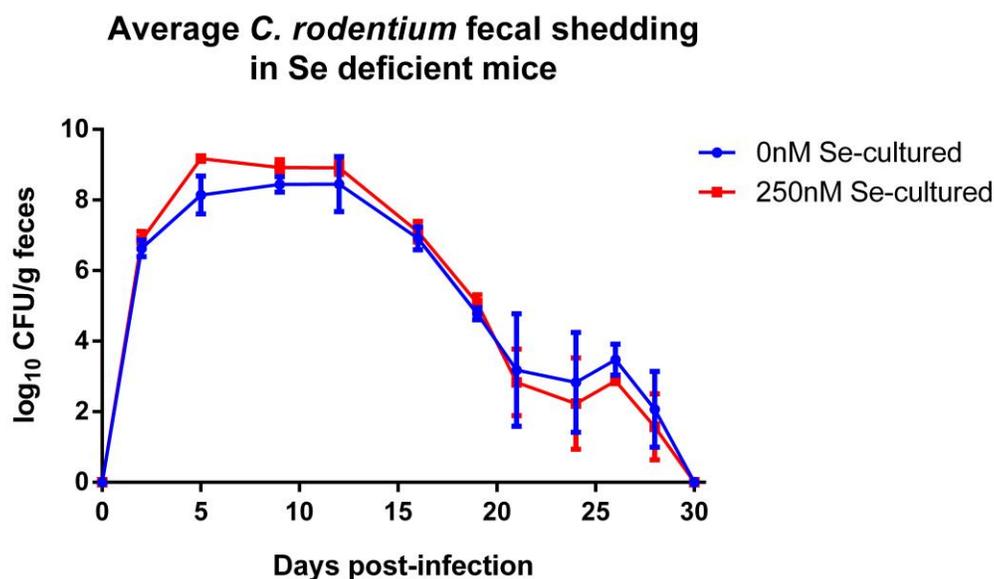


**Figure 3. Average bacterial fecal shedding for 0 nM and 250 nM Se-cultured *C. rodentium* in selenium deficient mice.** Mice (n=16) were each inoculated with 200μL ($5\times10^9$ CFU) of either 0 nM Se-cultured bacteria (n=8) or 250 nM Se-cultured bacteria (n=8).

**Selenium and DSS-induced colitis model**

Another murine model for gut inflammation is dextran sodium sulfate (DSS)-induced colitis, which mimics the pathology observed in humans with UC and induces a similar Th2 immune response (Viennois, E. et al., 2013; Neuman, M.G. and R.M. Nanau, 2012).

*Reducing the DSS interference in PCR*

Due to persistent downstream problems with amplification during PCR, the potential causes were investigated. Amplification was successfully attained for DNA isolated from non-DSS treated mice (Figure 4). This supports recent research that DSS inhibits downstream PCR (Viennois, E. et al., 2013).
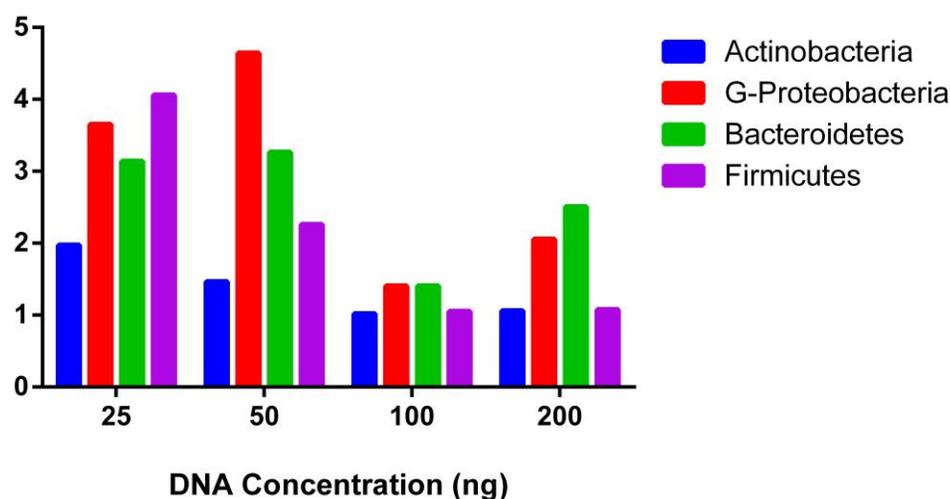


**Figure 4. Bacterial phyla in feces of non-DSS treated mice.** Two-fold dilutions were used to determine the best DNA concentration for PCR.

*DSS-induced colitis changes the gut microbiota*

Because DSS-induced colitis resembles some of characteristics seen in UC, changes in the murine gut microbiota throughout the DSS infection may indicate how the gut microbiome changes during an UC flare in patients.

One noticeable result was the absence of *Proteobacteria* until day 10, and even then there were still undetermined $C_T$ values for the *α-Proteobacteria* and *γ-Proteobacteria* phyla. Additionally, *Bacteroidetes* markedly increased in the selenium adequate mice by day 10, whereas only a marginal increased occurred in selenium supplemented mice (Figures 5and 6). The relative amount of *Actinobacteria* appeared to increase in selenium supplemented mice and stay relatively constant in selenium adequate mice. Furthermore, bacteria of the *Firmicutes* were most prevalent phyla in selenium adequate mice and least prevalent in selenium deficient mice. The $C_T$ values were almost all higher than those in selenium deficient mice, even the bacterial phyla associated with negative effects of inflammation.
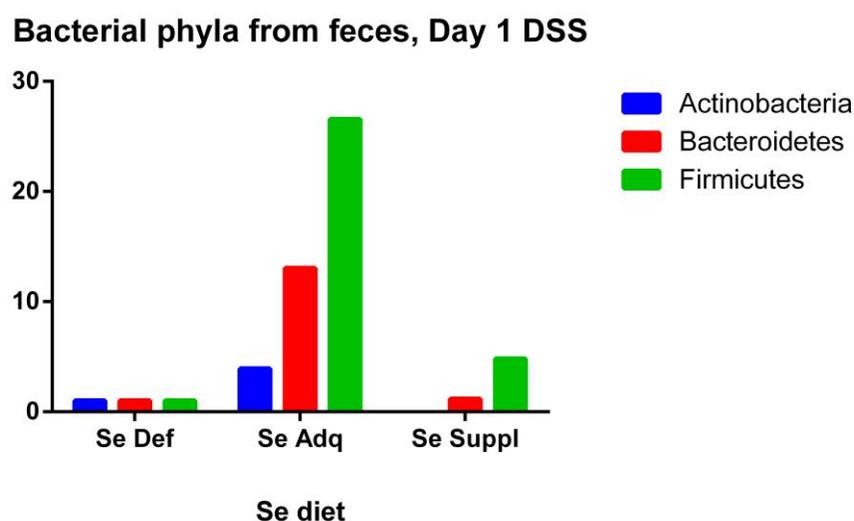


**Figure 5. Bacterial phyla present in gut on day 1 of DSS-induced colitis in mice.**
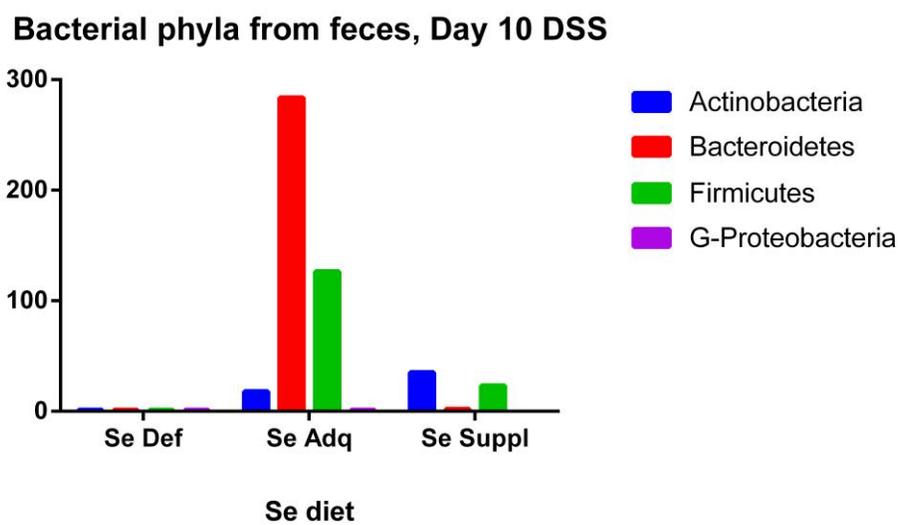
**Figure 6. Bacterial phyla present in gut on day 10 of DSS-induced colitis in mice.**

## Chapter 4

## Discussion and Conclusions

Previous studies in the laboratory have linked selenium with decreasing inflammation (Kaushal, N. et al., 2014; Nelson, S.M. et al., 2011). With sub-acute/chronic inflammation being one of the effects of IBD, the effects of selenium on gut inflammation were investigated.

For the *Citrobacter rodentium* experiments, the two groups of mice cleared their infections at the same time on average. But, while culturing *C. rodentium* in selenium deficient media did not decrease the duration of the infection, it did appear to make it less virulent, as the average number of CFU/g feces was lower during the peak of the infection.

For the DSS experiment, the results were surprising since other studies have observed a decrease in the *Bacteroidetes* phylum (Lupp, C. et al., 2007). The increase in *γ-Proteobacteria* after inflammation was induced with DSS agrees with results from similar studies (Lupp, C. et al., 2007). Furthermore, the results were surprising because they did not support the hypothesis that dietary selenium would help maintain the microbiota, as *Bacteroidetes* markedly increased by day 10 of the induced gut inflammation with DSS.

One explanation for this could be the purification of DNA with LiCl. After using two other purification procedures—Mo Bio PowerClean Pro DNA Clean-Up Kit and isopropanol—LiCl was used in order to obtain successful amplification with PCR. Viennois et al. provide evidence for the specific inhibition of downstream PCR due to DSS contamination in isolated RNA and their protocol was used to effectively remove it from these samples (Viennois, E. et al.,

2013). Since modifications to use this protocol for DNA could not be optimized, the exact protocol was followed, which may have also removed sufficient amounts of DNA along with the DSS. While subsequent PCR analysis showed successful amplification of the control primers, showing the LiCl procedure was successful in DSS removal, there may not have been enough DNA to get accurate $C_T$ values, as all of the bacterial primers still had "undetermined" $C_T$ values. This explanation coincides with the low DNA concentrations observed after the DSS removal. To solve this problem, RNA isolation with subsequent LiCl removal of DSS could be performed instead.

Ulcerative colitis and other forms of IBD remain a problem because of the expensive and individualized treatments that are often required to manage symptoms. More research into the etiology of IBD will help elucidate the next steps for preventive therapies. Future studies could look more in-depth into the effects of selenium and gut inflammation in both the *C. rodentium* and DSS models. Several further steps could be taken with the *C. rodentium* model, including examination of how the microbiota change throughout the course of the infection, looking at how selenium can maintain the gut microbiota due to inflammation from enteropathogenic infections, and whether supplementing mice with selenium only after being infected can minimize inflammation.

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## Education

The Pennsylvania State University—University Park, Pennsylvania Expected Graduation May 2015

    B.A. International Politics, International Relations concentration

    B.S. Immunology and Infectious Disease

    Minor in Global Health

## Honors/Awards

- Phi Beta Kappa Society      2015

- Penn State College of Agricultural Sciences Summer Research Grant      2014

- Pi Sigma Alpha Political Science Honor Society      2012

- Paterno Fellow      2011

- Schreyer Honors College Scholar      2010

## Research Experience

- Dr. K. Sandeep Prabhu's Lab, Undergraduate Researcher      2012-Present

## Clinical Volunteer Experience

- Mount Nittany Medical Center                                    2011-2014

- Penn State Milton S. Hershey Medical Center                     Summer 2012

- PinnacleHealth Harrisburg Hospital                              2008-2011

## Extracurricular Activities

- THON 2015 Public Relations Captain, Media Relations            2014-2015

- Global Brigades, Campus Council Executive Member               2013-2014

- THON 2014 Captain, Morale Committee                            2013-2014

- Homecoming 2013 Public Relations Captain, Media Relations      2013

- THON 2013 Committee Member                                     2012-2013

- Penn State Global Medical Brigades, Member                     2011-2013

- Student Red Cross Club, Member                                 2011-2013

- Homecoming 2012 Committee Member                               2012

## Internship Experience

- Intern for Pennsylvania State Representative Scott Conklin      Fall 2012